


# Follicle-stimulating hormone (FSH) receptor gene polymorphisms in Iraqi patients with non-obstructive azoospermia

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## ABSTRACT

**Background:** Follicle-stimulating hormone (FSH) is a pivotal hormone for male fertility, and its action on gonads is exerted by FSH receptors (FSHRs).

**Objectives:** To examine whether the presence of *FSHR* gene single nucleotide polymorphisms (SNPs), G919A and A2039G, involved in non-obstructive azoospermia (NOA) in Iraqi infertile men.

**Methods:** Two common SNPs, A919G and A2039G, in the *FSHR* gene were analyzed in 104 subjects (70 infertile patients with NOO: 33 NOA patients were not receiving treatment and 37 were on infertility treatment, and 34 normozoospermic fertile men as controls).

**Results:** The results revealed that the homozygous wild genotype (AA) of rs6165 *FSHR* gene SNP was more abundant than (AG) and (GG) genotypes in both groups of infertile NOA patients with a frequency of 49% in those who untreated, 81% in patients undergoing treatment and in the control group 41%. Whereas, the highest percentage of heterozygous genotype (AG) in the fertile control group was 41% when compared to NOA patients with a genotype frequency of 24% (for those who untreated) and 11% (for patients on treatment), respectively; with (A) allele frequency of 86% and the observed frequency of (G) allele was only 14% in the patients' group as compared to that of controls that were (65 %) and (35 %), respectively. The rs6166 genotyping revealed that the homozygous wild genotype (GG) of *FSHR* gene was more abundant than (AG) and (AA) genotypes in NOA patients receiving infertility treatment with a frequency of (68%), in NOA patients who didn't receive treatment 49%, while the lowest frequency was detected in the healthy fertile control group (47%).

**Conclusions:** These results support the evidence that rs6165 and rs6166, *FSHR* SNPs, might be involved in the pathogenesis and protection against NOA, respectively.

**Keywords** follicle-stimulating hormone receptor, male infertility, non-obstructive azoospermia, rs6165, rs6166, single nucleotide polymorphism

## INTRODUCTION

Infertility is a serious health problem, worldwide. Approximately 10-15% of couples (50-80 million people) within the reproductive age are suffering from the condition of infertility, according to the recently revealed data by the World Health Organization (WHO).<sup>1</sup> Males are about 50% of the reported infertility cases.<sup>2</sup> Azoospermia can be described as the absence of sperms in two separately collected semen samples following centrifugation (1000×g for about 15 min).<sup>3</sup> The etiology of azoospermia can be categorized into three principal classes: pre-testicular, testicular, and post-testicular azoospermia.<sup>4</sup> Azoospermic men comprise about 1% of the whole population and reach 10-20% of patients visiting infertility clinics.<sup>5</sup> In clinical workup, azoospermia can also be classified as either obstructive or non-obstructive.<sup>6</sup> The obstructive azoospermia (OA) conditions are less commonly reported than the non-obstructive azoospermia (NOA), giving rise to nearly 15-20% of the total infertile males with azoospermia.<sup>5</sup>

Gonadotrophins, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH), function as spermatogonial survival agents by controlling the intrinical apoptotic pathways.<sup>7</sup> Considering the FSH action as a pro-survival agent of germ cells, making alterations in the ratios of family protein members named as B-cell lymphoma 2 (BCL2), it acts through reducing apoptosis in the testis.<sup>7</sup> Medications comprised of FSH, the essential regulator of spermatogenesis, are administered empirically to manage male factor idiopathic infertility.<sup>8</sup> Genetic analysis could be, in this aspect, a possible candidate predictor to FSH treatment responsiveness concerning the opportunity to pregnancy.<sup>9</sup> Also, the single nucleotide polymorphism of the FSH receptor (*FSHR*) gene rs6166 (p.N680S SNP) has a reported effects on the ovarian response in females and testicular volume in adult males.<sup>7</sup>

The SNPs that appear in the *FSHR* gene can reduce FSH impact and be presented with different frequencies and allelic combinations in whole population. A clinical research manifested that the different *FSHR* genotypes affect FSH levels in serum and the gonadal responsiveness in both genders. Genotypes reducing FSH action are overrepresented in infertile subjects. Approximately 20% of the population were found to be carriers of alleles that were linked to reduced serum FSH concentrations/decreased *FSHR* expression levels or receptor activity, with lesser favorable trends for reproduction.<sup>10</sup> Findings of another study were revealed that the genetic polymorphisms of the *FSHR* gene were associated with a higher susceptibility to the incidence of azoospermia. Moreover, genetic polymorphisms within the *FSHR* gene (particularly; A919G and A2039G alleles) might affect FSH plasma level and spermatogenesis.<sup>11</sup>

Recently, the impact of *FSHR* gene polymorphisms of the two SNPs, G919A/A2039G in case of male infertility was noted. A study by Zhyilkova (2016)<sup>12</sup> found that 90% of azoospermia against 49% of non-azoospermic patients were reported to have FSH receptor gene polymorphisms. Moreover, a correlation was observed between FSH level and the existence of G919A alternative alleles among men diagnosed with NOA.

Thus, the current study aims to reveal, within Iraqi population, the genotype and allele frequency of *FSHR* gene polymorphisms (G919A and A2039G) among healthy normo-

zoospermic fertile and infertile patients with NOA and to assess their association with the incidence of this infertility condition.

## MATERIALS AND METHODS

### Study design and participants

This case-control study was conducted during the period of February 2019 to January 2020. Seventy infertile patients who matched our inclusion criteria (listed below) had been chosen for the study, in addition to thirty-four healthy normozoospermic fertile individuals were selected to be controls.

Male patients had been recruited from the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University (Baghdad, Iraq). The patients' clinical conditions and the azoospermia type were collected from their medical records, after nomination by urologist.

Firstly, the azoospermia was ascertained based on at least two separate seminal fluid analyses. Every participant had a clinical thorough workup, which was involved; the recorded clinical history, the physical examination, the endocrinology study added to the laboratory performed seminal fluid analysis (SFA) of the ejaculates. These examination results were pointed to this type of male infertility.

The patients' inclusion criteria for this study were: 1) male factor infertility for at least one year with an intact female partner, 2) at least two abnormal SFAs within six weeks demonstrating azoospermia according to WHO criteria,<sup>13</sup> and 3) minimally three months without receiving effective andrological treatment.

Exclusion criteria were: 1) any detectable infertility cause after conducting systemic physical examinations and laboratory investigations involving genetic disorders, 2) any diagnosed infectious diseases or immune-associated conditions, or the existence of any reported major systemic diseases, 3) thyroid gland dysfunction, adrenal abnormalities, overt hyperprolactinemia, added to any abnormal hormones related to the reproductive function, 4) abnormal psychological stresses, and 5) testicular surgeries, vasectomy or any other iatrogenic mass injuries to the reproductive tract of male had been excluded from the current study.

The present study was further approved by the Institutional Review Board (IRB) of the College of Medicine at Al-Nahrain University, in consistence with the Declaration of Helsinki (2013). Enrolment in the study had been done after obtaining informed consent from each participant.

### Seminal fluid analysis

Semen samples were obtained by masturbation preceded by an abstinence interval ranging from 2-7 days, near the laboratory for limiting the time separating the collection of

semen and analysis process. The physical properties of each semen sample, including; the volume, pH value, seminal fluid color, time of liquefaction, and seminal viscosity, were investigated, then the samples were examined under the microscope to evaluate the sperms' motility levels, vitality, concentration values, and the morphology. The collected values are then compared to the stated WHO manual's reference values.<sup>13</sup>

## Genetic analyses

### *The selection of candidate FSHR gene SNPs of the NOA*

The SNPs investigated in the current study were selected depending on the ClinVar, screening OMIM, and the SNPedia. They reported being SNPs with a higher susceptibility for NOA. In terms of hypothalamic-pituitary-gonadal (HPG) axis deregulating potential is associated with the pathophysiology of NOA (Table 1).

**Table 1** Nomenclature of the investigated gene and polymorphisms in the present study

Gene	Locus		refSNP	DNA nucleotide	Single-nucleotide variation	NCBI nucleotide reference sequence	Protein	NCBI protein reference sequence
<i>FSHR</i>	2p21 <i>Homo sapiens</i>	in	rs6165	c.919G>A	g.49191041C>'	N-T_022184.1	p.A307T	p23945.3
<i>FSHR</i>	2p21 <i>Homo sapiens</i>	in	rs6166	c.2039A>G	g.49189921T>('	N-T_022184.1	p.N680S	p23945.3

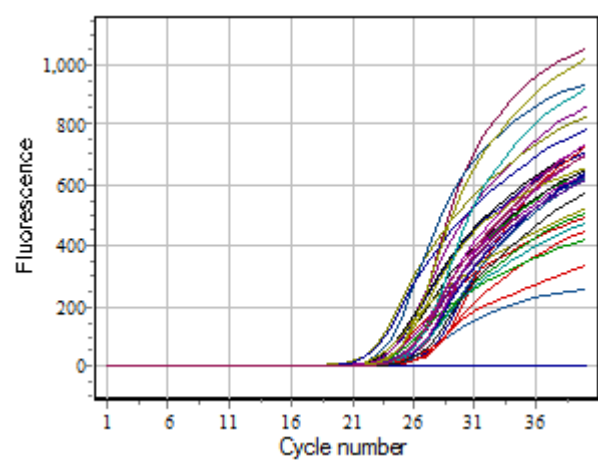
### *Extraction of genomic DNA from the whole blood*

QuicK-DNA™ Miniprep Kit (Catalog No. D3025, Zymo Research, USA) was utilized to purify DNA with high quality from samples of whole blood collected on EDTA-containing test tubes. The gained product was optimized for purity (DNA without any RNA contamination) and was compatible with the fresh or stored sample.

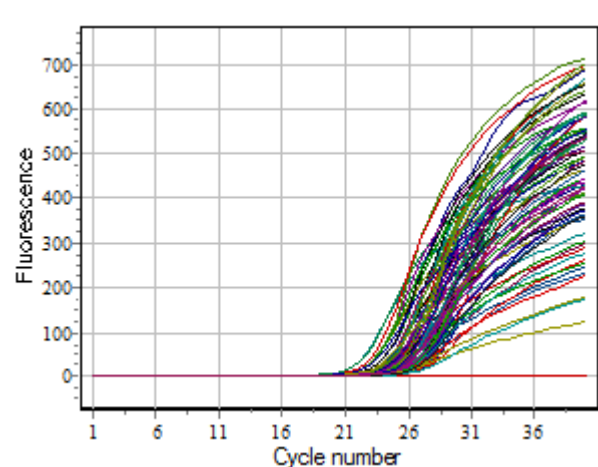
### *SNPs detection by quantitative real-time PCR*

According to the manufacturer's instructions, the genotyping of rs6165 *FSHR* genetic polymorphism was conducted using the real-time polymerase chain reaction (RT-PCR) technique (TaqMan™ SNP Genotyping Assay, human, Thermofisher Scientific, Cat. No. 4351379). The specific probe for the frequent gene allele (wild allele A) was marked with VIC reporter dye at the 5' end. The probe specified to the lesser frequent gene allele (mutated allele G) had been tagged with the FAM reporter dye at the 3' end. After reviewing the

amplification plot curves, the results were verified, as illustrated in Figure 1 and Figure 2.



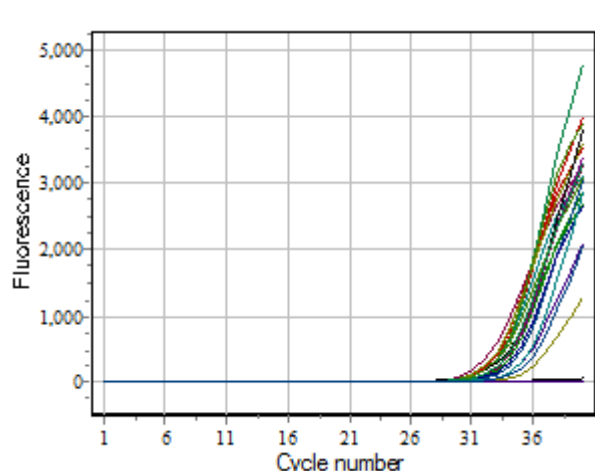
**Figure 1** Amplification plot curves dependence of VIC channel fluorescence on cycle number, representing A allele (rs6165).



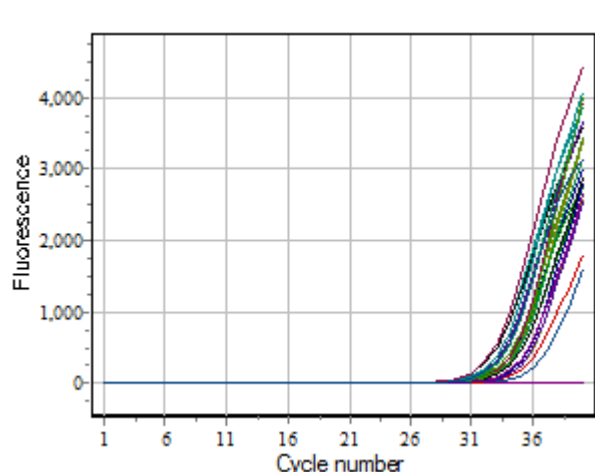
**Figure 2** Amplification plot curves dependence of FAM channel fluorescence on cycle number representing G allele (rs6165).

Genotyping of the rs6166 *FSHR* gene polymorphism was proceeded using RT-PCR according to the manufacturer instructions (TaqMa Genotyping Assay, human, ThermoFisher Scientific, Cat. No. 4351379). The probe specific for the much frequent gene allele (wild allele G) was tagged with VIC reporter dye at 5' end. In contrast, the probe specified for the lesser frequently distributed gene allele (mutated allele T) was marked with FAM reporter dye at 3' ends. The results were verified after reviewing the amplification plot curves as shown in Figure 3 and Figure 4.

The PCR amplification process conducted here was involved: An initial step performed at 94°C for 5 minutes; then followed by 30 cycles of denaturation at 94°C for the 30 seconds,



**Figure 3** Amplification plot curves dependence of VIC channel fluorescence on cycle number representing G allele (rs6166).



**Figure 4** Amplification plot curves dependence of FAM channel fluorescence on cycle number representing A allele (rs6166).

the DNA annealing at 55°C (for rs6165) or 60°C (for rs6166) for 30 seconds, then elongation process at 72°C for 1 minute and finally with the last extension at 72°C for about 10 minutes.

All subjects were categorized as being (AA) for the homozygous wild genotype of (rs6165) *FSHR* gene, (AG) for heterozygous polymorphism, and (GG) for homozygous mutated genotype. While, all subjects were categorized as being (GG) for the homozygous wild genotype of (rs6166) *FSHR* gene and (GA) for heterozygous polymorphism, and (AA) for homozygous mutated genotype.

### Statistical analysis

All analyses were carried out using SPSS (version 24) software (SPSS Inc., USA). Linear regression was used to compare the means of the investigated clinical parameters across the different genotypes (to compare each genotype's means, linear regression was done separately).

## RESULTS

Seventy infertile patients who presented with NOA collected in the present study were categorized into two groups according to their receiving infertility treatment (rFSH, rHCG, or HMG injection) or not. Thirty-three azoospermic patients not received treatment yet (patients group I) and 37 azoospermic patients receiving infertility treatment (patients group II), as well as 34 fertile healthy subjects as a control group (Table 2).

The mean  $\pm$  standard error of the mean (SEM) of age in years for the healthy control group was  $(33.59 \pm 1.222)$  and for the untreated infertile patient group (patients group I) with azoospermia=  $31.58 \pm 1.059$ ; and for infertile patients with azoospermia (patients group II) receiving treatment=  $33.46 \pm 1.173$  respectively. No significant variations ( $p > 0.05$ ) was found among the study groups.

The mean  $\pm$  SEM of body mass indices (BMIs) in kg/m<sup>2</sup> for the healthy fertile control group ( $26.67 \pm 0.6429$ ) and azoospermic infertile patient group not receiving treatment (patients group I) ( $26.17 \pm 0.8108$ ) and for those on treatment (patients group II) ( $28.70 \pm 0.919$ ) respectively, there were no significant differences ( $p > 0.05$ ) were found among them.

The mean  $\pm$  SEM of the duration of infertility in years for the azoospermic infertile patient group not receiving treatment (patients group I) was  $5.030 \pm 0.696$  and for patients group on treatment (patients group II) was  $6.378 \pm 0.505$  respectively, there were no statistically significant differences detected ( $p > 0.05$ ) between the two patients groups.

**Table 2** Age, body mass indexes and duration of infertility of different studied groups.

Groups	Age (years)	BMI (Kg/m <sup>2</sup> )	Duration of infertility (years)
Patient I	$31.58 \pm 1.059$	$26.17 \pm 0.8108$	$5.030 \pm 0.6966$
Patient II	$33.46 \pm 1.173$	$28.70 \pm 0.919$	$6.378 \pm 0.505$
Control	$33.59 \pm 1.222$	$26.67 \pm 0.6429$	-
P-value	0.995	0.175	0.116

**BMI**, body mass index; **Patient I**, azoospermic patients did not receive treatment; **Patient II**, azoospermic patients were receiving treatment.

## Genotyping of rs6165 FSHR gene

Statistical analysis revealed a significant variation in the distribution of the genotypes among different study groups. The homozygous wild genotype (AA) of the *FSHR* gene was more abundant than (AG) and (GG) genotypes in both groups of infertile azoosper-

mic patients with a frequency of (49%), (81%) and in the control group (41%) respectively. Whereas, the highest distribution frequency percentage of the heterozygous genotype (AG) in the healthy control group was (41%) as compared to patient groups with genotype frequency of (24%) and (11%) respectively, with an A allele frequency of distribution scored (86%) and G allele frequency of distribution of only (14%) in the patient group whether compared to that of control that approached (65%) and (35%) respectively. The data showed that GG carriers were less prevalent among men with non-obstructive azoospermia, which appeared clearly in (patients group II) with an allele frequency of 81% for AA, 11% for AG, and with only 8% for GG genotype carriers with a highly significant variation that scored  $p$ -value of 0.0048, as shown in Table 3.

**Table 3** Genotyping of rs6165 FSHR gene polymorphism with a different allele frequency distribution in the study groups.

Group	AA	AG	GG	A	G	Significant	Chi-squared	P - value	Total
Patient I	No. 16.00	8.00	9.00	40.00	26.00	*	7.998	0.0183	33
	% 49	24	27	60	40				
Patient II	No. 30.00	4.00	3.00	64.00	10.00	**	10.69	0.0048	37
	% 81	11	8	86	14				
Control	No. 15.00	14.00	5.00	44.00	24.00		0.3298	0.8480	34
	% 44	41	15	65	35				
Significance	**	*	NS.	**	**				
P-value	0.0024	0.0127	0.0914	0.0012	0.0012				
Total	61	26	17	148	60				

No., number; %, percent; NS, non-significant; \*, significant at the  $p$ -value  $\leq 0.05$ ; \*\*, significant at the  $p$ -value  $\leq 0.01$ ; **Patient I**, azoospermic patients did not receive treatment; **Patient II**, azoospermic patients were receiving treatment.

The significance of FSHR rs6165 G/A genotyping results was evaluated by utilizing the Chi-squared ( $\chi^2$ ) test. The odds ratios, the significance of genotyping distribution frequency, and the risk factors for the evolution of non-obstructive azoospermia in infertile patients groups compared with the healthy fertile control group. As shown in Table 4, the patients carrying homozygous wild AA genotype were at a significantly higher risk, while heterozygous AG genotype was significantly at a lower frequency in the patient groups in comparison to the fertile control group [(OR = 0.1842,  $P$  = 0.0012, CI: 0.0683 to 0.539) and (OR = 5.775,  $P$  = 0.003, CI: 1.75 to 17.44) respectively. Also, the data were shown that the G minor allele could be considered a protective factor in the patient group if compared to the control individuals (OR = 0.2865,  $P$  = 0.0024, CI: 0.126 to 0.657).

### Genotyping of rs6166 FSHR gene



**Table 4** Odds ratios, *p*-values, and confidence intervals of the *FSHR* rs6165 c.919G>A genotypes in various azoospermic infertile patient groups against the control group.

Comparison	Genotypes and alleles	<i>P</i> -value	OR	95% CI	
Patient I vs. control	Genotype	AA	0.72 <sup>NS</sup>	0.8388	0.314 to 2.2
		AG	0.14 <sup>NS</sup>	2.188	0.731 to 6.18
		GG	0.206 <sup>NS</sup>	0.4598	0.15 to 1.52
	Allele	A	0.6237 <sup>NS</sup>	1.192	0.5894 to 2.434
		G			
Patient II vs. control	Genotype	AA	0.0012 <sup>**</sup>	0.1842	0.0683 to 0.539
		AG	0.003 <sup>**</sup>	5.775	1.75 to 17.44
		GG	0.359 <sup>NS</sup>	2.011	0.46 to 8.03
	Allele	A	0.0024 <sup>**</sup>	0.2865	0.126 to 0.657
		G			

<sup>\*</sup>, significant at the *p*-value is  $\leq 0.01$ ; <sup>\*\*</sup>, significant at the *p*-value is  $\leq 0.05$ ; **CI**, confidence interval; **NS**, non-significant; **OR**, odd ratio; **Patient I**, azoospermic patients did not receive treatment; **Patient II**, azoospermic patients were receiving treatment.

Table (5) summarizes the allelic frequencies and genotype distributions of different study subjects. This table reveals that the homozygous wild genotype (GG) of the *FSHR* gene was more abundant than (AG) and (AA) genotypes in infertile azoospermic patients receiving infertility treatment (patients group II) with a frequency of (68 %), in infertile azoospermic patients who didn't receive treatment (patients group I) (49 %) and in the healthy fertile control group (47 %) respectively. In contrast, the lowest percentage of the distribution of heterozygous genotype (AG) in the healthy fertile control group was (17 %) with a scored G allele frequency of 57 % and an A allele frequency of only 43 %. Also, the highest percentage of frequency of distribution of the heterozygous genotype (AG) in infertile patients groups was (39 %) for (patients group I) and (32 %) for (patients group II) with a G allele frequency of (64 %) and (84 %) while for A allele frequency of (36 %) and (16 %) only, respectively. Statistical analysis revealed significant variation in the distribution of the genotypes among different study groups.

To evaluate the level of significance of *FSHR* rs6166 G/A genotyping distribution frequency results, the Chi-square test was manipulated to examine the odds ratios, the significance of the distribution of genotyping, and the risk factors for the incidence of azoospermia in the infertile patient's group in comparison to the healthy fertile individuals in the control group. As illustrated in the Table 6, the patients whose heterozygous AG genotype carriers were at a higher significant risk than homozygous AA genotype to have azoospermia in (patients group I) of the infertile patient whether compared with the control healthy subjects OR = 0.2755, *P* = 0.016, CI: 0.101 to 0.84. Furthermore, both infertile patient groups (patient groups I and II) had shown a significantly lower frequency of homozygous AA genotype in comparison to that of the healthy fertile control group with OR = 3.467, *P* = 0.047, CI: 0.963 to 10.8 for (patients group I) and *p*-value = 0.0002 for (patients group II) when compared with control, what accentuate the importance of the minor A allele as a protective factor against the incidence of male factor infertility presented with azoospermia.

**Table 5** Genotyping of rs6166 FSHR gene polymorphism with adifferent allele frequency distribution in the study groups.

Group	GG	AG	AA	G	A	Significa	Chi-squared	P-value	Total	
Patient I	No.	16.00	13.00	4.00	42.00	24.00	NS.	0.07482	0.9633	33
	%	49	39	12	64	36				
Patient II	No.	25.00	12.00	0.00	62.00	12.00	NS.	1.386	0.5001	37
	%	68	32	0	84	16				
Control	No.	16.00	6.00	12.00	39.00	29.00	**	11.40	0.0033	34
	%	47	17	36	57	43				
Significa	*	*	**	**	**					
P-value	0.0492	0.0474	0.0005	0.0017	0.0017					
Total	57	31	16	143	65					

No., number; %, percent; NS, non-significant; \*, significant at the  $p$ -value  $\leq 0.05$ ; \*\*, significant at the  $p$ -value  $\leq 0.01$ ; **Patient I**, azoospermic patients did not receive treatment; **Patient II**, azoospermic patients were receiving treatment.

**Table 6** Odds ratios,  $p$ -values, and confidence intervals of the FSHR rs6166c.2039A>G genotypes in various azoospermic infertile patient groups against the control group.

Comparison	Genotypes and alleles	P-value	OR	95% CI
Patient I vs. control	Genotype GG	0.527 <sup>NS</sup>	1.368	0.517 to 3.77
	Genotype AG	0.016*	0.2755	0.101 to 0.84
	Genotype AA	0.047*	3.467	0.963 to 10.8
	Allele G	0.457 <sup>NS</sup>	0.7685	0.3798 to 1.527
	Allele A			
Patient II vs. control	Genotype GG	0.081 <sup>NS</sup>	0.4267	0.171 to 1.09
	Genotype AG	0.260 <sup>NS</sup>	0.5401	0.198 to 1.49
	Genotype AA	0.0002**	-	-
	Allele G	0.0005**	0.2603	0.1161 to 0.5874
	Allele A			

\*, significant at the  $p$ -value  $\leq 0.01$ ; \*\*, significant at the  $p$ -value  $\leq 0.05$ ; CI, confidence interval; NS, non-significant; OR, odds ratio; **Patient I**, azoospermic patients did not receive treatment; **Patient II**, azoospermic patients were receiving treatment.

## DISCUSSION

The *FSHR* gene consists of nine introns and ten exons and a promoter region that is localized on chromosome 2p21. The binding of FSH to its cell-surface follicle-stimulating hormone receptor mainly expressed on the Sertoli cells will initiate signaling, which ultimately stimulates the proliferation of these cells, so maintaining normal sperm production (spermatogenesis). Furthermore, the intercellular communications of the Sertoli cells with the germ cells in the vicinity added to other somatic cells will be promoted. Moreover, the FSH action aids in defining the testicular size and determination of germ cell count in testes. The *FSHR* gene contains multiple single-nucleotide variations or SNPs located either within the core promoter or the coding regions. Various research of male infertility causation was investigated the possible association between the different Thr307Ala and Asn680Ser single nucleotide polymorphisms in the *FSHR* gene and the development of the condition.<sup>13</sup>

A concomitant evaluation of the association of *FSHR* gene polymorphisms (Thr307Ala and Asn680Ser) with the possible risks of male factor infertility presented as an absence of sperms in the seminal fluid sample (non-obstructive azoospermia) were conducted in the current study.

The present study was revealed a statistically significant variation in the distribution of genotypes regarding rs6165 *FSHR* gene SNP, whether AA, AG or GG genotype carriers of the study azoospermic patients enrolled in comparison with the distribution of genotypes carriers in individuals of the fertile normozoospermic group that revealed a non-significantly varied distribution of the three genotypes. This variation in the genotypic distribution in infertile men was observed to be close to the results evoked by other findings as that revealed by Safarinejad and colleagues on a sample of the Iranian population<sup>14</sup> and Shimoda et al.<sup>15</sup> on a sample of the Japanese population. This pattern of a different distribution of genotypes in normozoospermic and azoospermic infertile men, and that the alleles of A-Ala-Ser and the G-Thr-Asn of *FSHR* gene rs6165 SNP were also reported by Ahda et al.<sup>16</sup> that were also evaluated this kind of genetic polymorphisms within the German population, despite the authors found that the *FSHR* haplotypes did not look to be associated with the various concentrations of the serum FSH in the infertile patient. Still, they concluded that the various genotypic distribution could present a contributing genetic factor for the severe spermatogenic impairment of phenotypic expressions.

On the other hand, these findings are opposite to that of studies performed by various researchers, who observed no difference in the frequency distribution of this single nucleotide polymorphism between infertile and healthy control cohorts, of these authors; Tuerlings and colleagues<sup>17</sup> and Simoni and colleagues<sup>18</sup> whose traced the influence of Ala307Thr and Asn680Ser polymorphism of the *FSHR* gene in the Dutch and the German patients' samples respectively. The findings demonstrated no difference in genotypic distribution. They led them to conclude that the mutations of the *FSHR* gene do not seem to exert pathogenic contributions to male factor idiopathic infertility.

This study also revealed a statistically significant increase in genotypic frequency with a higher abundance of wild type AA and heterozygous polymorphic AG genotype with a

much more clear picture of this type of distribution frequency of rs6165 *FSHR* gene SNP in group patients II of infertile men presented with non-obstructive azoospermia, whether compared to the distribution in healthy control fertile group. This set of findings follows findings collected elsewhere on various populations.<sup>19,20</sup> These studies adopted the opinion that state; *FSHR* polymorphisms may have an impact on reproductive function in men: *FSHR* 2039A>G, especially when associated with an SNP in the FSH  $\beta$  gene (c.-211G > T) was significantly associated with elevated serum FSH, lower testicular volume and diminished sperm count. Other researchers reported that *FSHR* p.Asn680Ser single nucleotide polymorphism had been associated with minimal response to the empirical treatment with FSH in male idiopathic oligozoospermia and infertility.<sup>7,21</sup>

The current study of *FSHR* rs6166 SNP genotyping frequency of the different study individuals revealed that the homozygous wild genotype (GG) of the *FSHR* gene was more abundant than (AG) and (AA) genotypes in infertile azoospermic patients receiving infertility treatment (patients group II) with a frequency of (68%), in infertile azoospermic patients who didn't receive treatment (patients group I) (49%). In comparison, the lowest frequency was detected in the healthy fertile control group (47%). These collected findings had agreed with previous studies,<sup>22,23</sup> were also revealed robust associations between the existence of FSHb (\_211G>T) and each of *FSHR* (Asn680Ser and Thr307Ala) polymorphisms with the response to the treatment. These studies demonstrated that the patients carrying the FSHb and *FSHR* mutated alleles exhibited significantly much greater improvements of the different classical seminal parameters, including; total sperms count, the motility, and morphology of sperms when compared to that of the patients of the wild genotype carriers.

Other studies corroborated the negligible associations among the different *FSHR* haplotypes and the extensively impaired spermatogenesis or azoospermia status. An Estonian study, where the G-29A, A919G, and A2039G common polymorphisms within the *FSHR* gene were analyzed in 150 (36 with non-obstructive azoospermia and another 114 presented with oligozoospermia) patients in addition to 208 healthy fertile normozoospermic males. Their results shown that the *FSHR* gene polymorphisms were not correlated to either the azoo- or the oligozoospermic conditions, which were prevailed from the similarity of the alleles, the genotypes, and the distribution of the haplotypes among the infertile patients and the controls, leading them to suggest that; the *FSHR* genetic haplotypes are excluded from being a considerable risk factor regarding the failure of spermatogenesis. Despite this, they stated that the G-Thr-Asn haplotypes were more abundant among the normozoospermic adult males than in the azoospermia infertile patients, leading them to emphasize a possible protective character of the studied haplotypes.<sup>24</sup>

The SNPs lying within the *FSHR* gene may give rise to modulated receptor expression and signaling.<sup>8,25</sup> The most studied *FSHR* polymorphisms, from more than 2000 described, are: p.Thr307Ala variant (c.919 A > G; rs6165)<sup>8</sup> which produces a change of the threonine amino acid to the alanine amino acid at the position of 307 at the hinge region of the follicle-stimulating hormone receptor. This SNP resides on exon 10 in a linkage disequilibrium state with the p.Asn680Ser (c.2039A > G; rs6166) variant, which changes asparagine amino acid to serine amino acid localized at the intracellular domain of the FSHR. Further-

more, the existing serine amino acid results in differently induced kinetics of each cAMP, the pERK1/2, and the activation of the pCREB cascade.<sup>26</sup> So it expected to result in an aberrated receptor stimulation by their ligands (FSH) and interfering with the proper endocrine function necessary for the optimized process of spermatogenesis.

The discrepancy between various studies exhibited above can be attributed to ethnic and geographic variation populations, making it challenging to establish ethnicity since most of the populations are mixed.<sup>21</sup> Furthermore, the sizes of the studied samples are interesting attributes to be considered in interpreting the results, making it the main limitation of our findings. Moreover, it is unfair to make data associations from small sample size studies with varied patients' inclusion criteria and ethnically divergent populations.

## CONCLUSIONS

An increase in the frequency of homozygotes of wild type AA and heterozygous polymorphic AG genotype of distribution frequency of rs6165 *FSHR* gene SNP compared to that theoretically expected and homozygous wild genotype (GG) of rs6166 *FSHR* gene SNP was more abundant than (AG) and (AA) genotypes in patients concerning the control was observed. The results indicate the probable pathogenic role of the wild genotype and the protective role of the mutant minor one. So results shed light on the hidden role of the *FSHR* gene SNPs in the etiology of NOA along with other multiple genetic-environmental contributing factors, suggesting further research on a larger scale in terms of sample size for NOA and for other forms of azoospermia to unveil its genetic heterogeneity and the establishing of strategies for the management of male infertility.

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## DECLARATIONS

### Authors' contributions

Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing-original draft, and writing-review & editing: AAA, OFA and UMA. Funding acquisition: N/A. Supervision: OFA and UMA. All authors have read, reviewed and approved the final version before publication.

### Conflict of interest

The authors state that there are no conflicts of interest to disclose.

## Ethical approvals

The Institutional Review Board of the College of Medicine, Al-Nahrain University, Baghdad, Iraq approved all experimental procedures and protocols (No.: IRB/160, Date: 2018-09-04). Written informed consent was obtained from patients themselves or legally authorized representative(s) for anonymized patient information to be published in this article.

## Data availability

Data associated with this paper can be requested from the corresponding author upon reasonable request.

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